Efficient genome searching with Biostrings and the BSgenome data packages

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June 30, 2024

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1 The Biostrings-based genome data packages

The Bioconductor project provides data packages that contain the full genome sequences of a given organism. These packages are called *Biostrings-based genome data packages* because the sequences they contain are stored in some of the basic containers defined in the Biostrings package, like the *DNAString*, the *DNAStringSet* or the *MaskedDNAString* containers. Regardless of the particular sequence data that they contain, all the Biostrings-based genome data packages are very similar and can be manipulated in a consistent and easy way. They all require the BSgenome package in order to work properly. This package, unlike the Biostrings-based genome data packages are also called *BSgenome data packages*). The BSgenome package itself requires the Biostrings package.

See the man page for the available.genomes function (?available.genomes) for more information about how to get the list of all the BS genome data packages currently available in your version of Bioconductor (you need an internet connection so that available.genomes can query the Bioconductor package repositories).

Note that the BSgenomeForge package provides tools that you can use to make your own BSgenome data package.

2 Finding an arbitrary nucleotide pattern in a chromosome

In this section we show how to find (or just count) the occurences of some arbitrary nucleotide pattern in a chromosome. The basic tool for this is the matchPattern (or countPattern) function from the Biostrings package.

First we need to install and load the BS genome data package for the organism that we want to look at. In our case, we want to search chromosome I of *Caenorhabditis elegans*.

UCSC provides several versions of the C. elegans genome: ce1, ce2 and ce4. These versions correspond to different *releases* from WormBase, which are the WS100, WS120 and WS170 releases, respectively. See http://genome.ucsc.edu/FAQ/FAQreleases#release1 for the list of all UCSC genome releases and for the correspondance between UCSC versions and release names.

The BS genome data package for the ce2 genome is BS genome.Celegans.UCSC.ce2. Note that ce1 and ce4 are not available in Bioconductor but they could be added if there is demand for them.

See ?available.genomes for how to install BSgenome.Celegans.UCSC.ce2. Then load the package and display the single object defined in it:

```
> library(BSgenome.Celegans.UCSC.ce2)
> ls("package:BSgenome.Celegans.UCSC.ce2")
[1] "BSgenome.Celegans.UCSC.ce2" "Celegans"
> genome <- BSgenome.Celegans.UCSC.ce2</pre>
> genome
BSgenome object for Worm
| - organism: Caenorhabditis elegans
- provider: UCSC
- genome: ce2
- release date: Mar. 2004
- 7 sequence(s):
      chrI
             chrII chrIII chrIV chrV
                                         chrX
                                                 chrM
| Tips: call 'seqnames()' on the object to get all the sequence names, call
| 'seqinfo()' to get the full sequence info, use the '$' or '[[' operator to
| access a given sequence, see '?BSgenome' for more information.
  genome is a BSgenome object:
```

> class(genome)

[1] "BSgenome" attr(,"package") [1] "BSgenome"

When displayed, some basic information about the origin of the genome is shown (organism, genome, provider, etc...) followed by the index of *single* sequences and eventually an additional index of *multiple* sequences. Methods (adequately called *accessor methods*) are defined for individual access to this information:

```
> metadata(genome)
```

```
$organism
[1] "Caenorhabditis elegans"
```

\$common_name

[1] "Worm" \$provider [1] "UCSC" \$genome [1] "ce2" \$release_date [1] "Mar. 2004" \$source url [1] "http://hgdownload.cse.ucsc.edu/goldenPath/ce2/bigZips/" > seqnames(genome) [1] "chrI" "chrII" "chrIII" "chrIV" "chrV" "chrX" "chrM" > seqinfo(genome) Seqinfo object with 7 sequences (1 circular) from ce2 genome: seqnames seqlengths isCircular genome chrI15080483 FALSE ce2 chrII FALSE 15279308 ce2 chrIII 13783313 FALSE ce2 chrIV 17493791 FALSE ce2 chrV 20922231 FALSE ce2 chrX 17718849 FALSE ce2 chrM13794 TRUE ce2

See the man page for the *BSgenome* class (?BSgenome) for a complete list of accessor methods and their descriptions.

Now we are ready to display chromosome I:

> genome\$chrI

```
15080483-letter DNAString object
seq: GCCTAAGCCTAAGCCTAAGCCTAAGCCTAAGCCTAAGCCTAAGGCTTAGGCTTAGGCTTAGGCTTAGGCTTAGGCTTAGGCTTAGGCTTAGGC
```

Note that this chrI sequence corresponds to the *forward* strand (aka *direct* or *sense* or *positive* or *plus* strand) of chromosome I. UCSC, and genome providers in general, don't provide files containing the nucleotide sequence of the *reverse* strand (aka *indirect* or *antisense* or *negative* or *minus* or *opposite* strand) of the chromosomes because these sequences can be deduced from the *forward* sequences by taking their reverse complements. The BSgenome data packages are no exceptions: they only provide the *forward* strand sequence of every chromosome. See **?reverseComplement** for more details about the reverse complement of a *DNAString* object. It is important to remember that, in practice, the *reverse* strand sequence is almost never needed. The reason is that, in fact, a *reverse* strand analysis can (and should) always be transposed into a *forward* strand analysis. Therefore trying to compute the *reverse* strand sequence of an entire chromosome by applying **reverseComplement** to its *forward* strand sequence is almost always a bad idea. See the *Finding an arbitrary nucleotide pattern in an entire genome* section of this document for how to find arbitrary patterns in the *reverse* strand of a chromosome.

The number of bases in this sequence can be retrieved with:

```
> chrI <- genome$chrI</pre>
> length(chrI)
[1] 15080483
   Some basic stats:
> afI <- alphabetFrequency(chrI)</pre>
> afI
               С
                        G
                                 Т
                                         М
                                                  R
                                                           W
                                                                    S
                                                                             Y
                                                                                      Κ
      А
4838561 2697177 2693544 4851201
                                         0
                                                  0
                                                           0
                                                                    0
                                                                             0
                                                                                      0
      V
               Η
                        D
                                В
                                         Ν
                                                  _
                                                           +
      0
               0
                        0
                                0
                                         0
                                                  0
                                                           0
                                                                    0
```

```
> sum(afI) == length(chrI)
```

[1] TRUE

Count all *exact* matches of pattern "ACCCAGGGC":

```
> p1 <- "ACCCAGGGC"
> countPattern(p1, chrI)
```

[1] 0

Like most pattern matching functions in Biostrings, the countPattern and matchPattern functions support *inexact* matching. One form of inexact matching is to allow a few mismatching letters per match. Here we allow at most one:

```
> countPattern(p1, chrI, max.mismatch=1)
```

[1] 235

With the matchPattern function, the locations of the matches are stored in an XStringViews object:

```
> m1 <- matchPattern(p1, chrI, max.mismatch=1)</pre>
> m1[4:6]
Views on a 15080483-letter DNAString subject
subject: GCCTAAGCCTAAGCCTAAGCCTAAGCCTAAGCCT...AGGCTTAGGCTTAGGCTTAGGCTTAGGCTTAGGC
views:
       start
                end width
  [1] 187350 187358 9 [ACCCAAGGC]
  [2] 213236 213244
                       9 [ACCCAGGGG]
                       9 [ACCCAGGAC]
  [3] 424133 424141
> class(m1)
[1] "XStringViews"
attr(,"package")
[1] "Biostrings"
```

The mismatch function (new in Biostrings 2) returns the positions of the mismatching letters for each match:

```
> mismatch(p1, m1[4:6])
[[1]]
[1] 6
[[2]]
[1] 9
[[3]]
[1] 8
```

Note: The **mismatch** method is in fact a particular case of a (vectorized) *alignment* function where only "replacements" are allowed. Current implementation is slow but this will be addressed.

It may happen that a match is *out of limits* like in this example:

```
> p2 <- DNAString("AAGCCTAAGCCTAAGCCTAA")
> m2 <- matchPattern(p2, chrI, max.mismatch=2)</pre>
> m2[1:4]
Views on a 15080483-letter DNAString subject
subject: GCCTAAGCCTAAGCCTAAGCCTAAGCCTAAGCCT...AGGCTTAGGCTTAGGCTTAGGCTTAGGCTTAGGC
views:
      start end width
  [1]
         -1 18
                   20 [ GCCTAAGCCTAAGCCTAA]
  [2]
          5 24
                   20 [AAGCCTAAGCCTAAGCCTAA]
  [3]
         11 30
                   20 [AAGCCTAAGCCTAAGCCTAA]
  [4]
         17 36
                   20 [AAGCCTAAGCCTAAGCCTAA]
> p2 == m2[1:4]
[1] FALSE TRUE TRUE TRUE
> mismatch(p2, m2[1:4])
[[1]]
[1] 1 2
[[2]]
integer(0)
[[3]]
integer(0)
[[4]]
integer(0)
```

The list of exact matches and the list of inexact matches can both be obtained with:

> m2[p2 == m2] > m2[p2 != m2]

Note that the length of m2[p2 == m2] should be equal to countPattern(p2, chrI, max.mismatch=0).

3 Finding an arbitrary nucleotide pattern in an entire genome

Now we want to extend our analysis to the *forward* and *reverse* strands of all the C. elegans chromosomes. More precisely, here is the analysis we want to perform:

- The input dictionary: Our input is a dictionary of 50 patterns. Each pattern is a short nucleotide sequence of 15 to 25 bases (As, Cs, Gs and Ts only, no Ns). It is stored in a FASTA file called "ce2dict0.fa". See the *Finding all the patterns of a constant width dictionary in an entire genome* section of this document for a very efficient way to deal with the special case where all the patterns in the input dictionary have the same length.
- The target: Our target (or subject) is the *forward* and *reverse* strands of the seven C. elegans chromosomes (14 sequences in total). We want to find and report all occurences (or hits) of every pattern in the target. Note that a given pattern can have 0, 1 or several hits in 0, 1 or 2 strands of 0, 1 or several chromosomes.
- Exact or inexact matching? We are interested in exact matches only (for now).
- The output: We want to put the results of this analysis in a file so we can send it to our collaborators for some post analysis work. Our collaborators are not necessarily familiar with R or Bioconductor so dumping a high-level R object (like a list or a data frame) into an .rda file is not an option. For maximum portability (one of our collaborators wants to use Microsoft Excel for the post analysis) we choose to put our results in a tabulated file where one line describes one hit. The columns (or fields) of this file will be (in this order):
 - seqname: the name of the chromosome where the hit occurs.
 - start: an integer giving the starting position of the hit.
 - end: an integer giving the ending position of the hit.
 - strand: a plus (+) for a hit in the positive strand or a minus (-) for a hit in the negative strand.
 - patternID: we use the unique ID provided for every pattern in the "ce2dict0.fa" file.

Let's start by loading the input dictionary with:

```
> ce2dict0_file <- system.file("extdata", "ce2dict0.fa", package="BSgenome")
> ce2dict0 <- readDNAStringSet(ce2dict0_file, "fasta")
> ce2dict0
```

DNAStringSet object of length 50:

	width	seq	names
[1]	18	GCGAAACTAGGAGAGGCT	pattern01
[2]	25	CTGTTAGCTAATTTTAAAAAATAAAT	pattern02
[3]	24	ACTACCACCCAAATTTAGATATTC	pattern03
[4]	24	AAATTTTTTTTTGTTGCAAATTTGA	pattern04
[5]	25	TCTTCTTGGCTTTGGTGGTACTTTT	pattern05
		•••	
[46]	24	TTTTGAACAAAGCATGTCTAACTA	pattern46
[47]	20	TAAACGAATTTAGGATATAT	pattern47
[48]	19	AAGGACCAGGATTGGCACG	pattern48
[49]	24	AAATAACTGCGTAAAAACACAATA	pattern49
[50]	22	AAAATGCCGGAGCATTTTAAAG	pattern50

Here is how we can write the functions that will perform our analysis:

```
> writeHits <- function(seqname, matches, strand, file="", append=FALSE)
+ {
+
      if (file.exists(file) && !append)
          warning ("existing file ", file, " will be overwritten with 'append=FALSE'")
+
+
      if (!file.exists(file) && append)
          warning("new file ", file, " will have no header with 'append=TRUE'")
+
+
      hits <- data.frame(seqname=rep.int(seqname, length(matches)),</pre>
+
                           start=start(matches),
+
                           end=end(matches),
+
                           strand=rep.int(strand, length(matches)),
+
                           patternID=names(matches),
+
                           check.names=FALSE)
      write.table(hits, file=file, append=append, quote=FALSE, sep="\t",
+
                   row.names=FALSE, col.names=!append)
+
+ }
> runAnalysis1 <- function(dict0, outfile="")</pre>
+ {
+
      library(BSgenome.Celegans.UCSC.ce2)
      genome <- BSgenome.Celegans.UCSC.ce2
+
+
      seqnames <- seqnames(genome)</pre>
      seqnames_in1string <- paste(seqnames, collapse=", ")</pre>
+
+
      cat("Target:", metadata(genome)$genome,
+
           "chromosomes", seqnames_in1string, "\n")
+
      append <- FALSE
+
      for (seqname in seqnames) {
+
          subject <- genome[[seqname]]</pre>
+
           cat(">>> Finding all hits in chromosome", seqname, "...\n")
+
          for (i in seq_len(length(dict0))) {
              patternID <- names(dict0)[i]</pre>
+
              pattern <- dict0[[i]]</pre>
+
+
              plus_matches <- matchPattern(pattern, subject)</pre>
              names(plus_matches) <- rep.int(patternID, length(plus_matches))</pre>
+
               writeHits(seqname, plus_matches, "+", file=outfile, append=append)
+
+
               append <- TRUE
+
               rcpattern <- reverseComplement(pattern)</pre>
               minus_matches <- matchPattern(rcpattern, subject)</pre>
+
+
              names(minus_matches) <- rep.int(patternID, length(minus_matches))</pre>
               writeHits(seqname, minus_matches, "-", file=outfile, append=append)
+
+
          7
+
          cat(">>> DONE\n")
+
      }
+ }
```

Some important notes about the implementation of the runAnalysis1 function:

- subject <- genome[[seqname]] is the code that actually loads a chromosome sequence into memory. Using only one sequence at a time is a good practice to avoid memory allocation problems on a machine with a limited amount of memory. For example, loading all the human chromosome sequences in memory would require more than 3GB of memory!
- We have 2 nested for loops: the outer loop walks thru the target (7 chromosomes) and the inner loop walks thru the set of patterns. Doing the other way around would be very inefficient, especially with a

bigger number of patterns because this would require to load each chromosome sequence into memory as many times as the number of patterns. **runAnalysis1** loads each sequence only once.

• We find the matches in the minus strand (minus_matches) by first taking the reverse complement of the current pattern (with rcpattern <- reverseComplement(pattern)) and NOT by taking the reverse complement of the current subject.

Now we are ready to run the analysis and put the results in the "ce2dict0_ana1.txt" file:

```
> runAnalysis1(ce2dict0, outfile="ce2dict0_ana1.txt")
```

```
Target: ce2 chromosomes chrI, chrII, chrIII, chrIV, chrV, chrX, chrM
>>> Finding all hits in chromosome chrI ...
>>> DONE
>>> Finding all hits in chromosome chrII ...
>>> DONE
>>> Finding all hits in chromosome chrIV ...
>>> DONE
>>> Finding all hits in chromosome chrIV ...
>>> DONE
>>> Finding all hits in chromosome chrV ...
>>> DONE
>>> Finding all hits in chromosome chrV ...
>>> DONE
>>> Finding all hits in chromosome chrV ...
>>> DONE
>>> Finding all hits in chromosome chrV ...
>>> DONE
>>> Finding all hits in chromosome chrV ...
>>> DONE
>>> Finding all hits in chromosome chrX ...
>>> DONE
>>> DONE<
>>> Finding all hits in chromosome chrX ...
>>> DONE<
>>> DONE
```

Here is some very simple example of post analysis:

• Get the total number of hits:

```
> hits1 <- read.table("ce2dict0_ana1.txt", header=TRUE)
> nrow(hits1)
```

[1] 79

- Get the number of hits per chromosome:
 - > table(hits1\$seqname)

chrI chrII chrIII chrIV chrM chrV chrX 11 5 16 8 8 15 16

• Get the number of hits per pattern:

```
> hits1_table <- table(hits1$patternID)
> hits1_table
```

pattern01 pattern02 pattern03 pattern04 pattern06 pattern07 pattern08 pattern09 1 1 2 1 1 1 1 1 pattern10 pattern11 pattern12 pattern13 pattern14 pattern15 pattern16 pattern17 1 1 1 1 1 1 1 1 pattern18 pattern19 pattern20 pattern21 pattern22 pattern23 pattern24 pattern25 2 1 9 1 10 1 1 1 pattern26 pattern27 pattern28 pattern29 pattern30 pattern31 pattern32 pattern33

```
1
                            1
                                       1
                                                 1
                                                           1
                                                                      1
                  1
                                                                                1
pattern34 pattern35 pattern36 pattern37 pattern38 pattern39 pattern40 pattern41
                                       7
        2
                  1
                            1
                                                 1
                                                           1
                                                                      1
                                                                                1
pattern42 pattern43 pattern44 pattern45 pattern46 pattern47 pattern48 pattern49
                  5
                                       1
                                                           1
        1
                            1
                                                 1
                                                                      1
                                                                                1
pattern50
        1
```

• Get the pattern(s) with the higher number of hits:

```
> hits1_table[hits1_table == max(hits1_table)] # pattern(s) with more hits
```

pattern21 10

• Get the pattern(s) with no hits:

```
> setdiff(names(ce2dict0), hits1$patternID) # pattern(s) with no hits
```

```
[1] "pattern05"
```

• And finally a function that can be used to plot the hits:

```
> plotGenomeHits <- function(bsgenome, seqnames, hits)</pre>
+ {
      chrlengths <- seqlengths(bsgenome)[seqnames]</pre>
+
      XMAX <- max(chrlengths)</pre>
+
      YMAX <- length(seqnames)
+
      plot.new()
+
      plot.window(c(1, XMAX), c(0, YMAX))
+
      axis(1)
+
      axis(2, at=seq_len(length(seqnames)), labels=rev(seqnames), tick=FALSE, las=1)
+
+
      ## Plot the chromosomes
+
      for (i in seq_len(length(seqnames)))
+
          lines(c(1, chrlengths[i]), c(YMAX + 1 - i, YMAX + 1 - i), type="1")
+
      ## Plot the hits
+
      for (i in seq_len(nrow(hits))) {
          seqname <- hits$seqname[i]</pre>
+
          y0 <- YMAX + 1 - match(seqname, seqnames)
+
          if (hits$strand[i] == "+") {
+
+
               y < -y0 + 0.05
               col <- "red"
+
          } else {
+
              y <- y0 - 0.05
+
               col <- "blue"</pre>
+
+
          7
+
          lines(c(hits$start[i], hits$end[i]), c(y, y), type="1", col=col, lwd=3)
      }
+
+ }
```

Plot the hits found by runAnalysis1 with:

```
> plotGenomeHits(genome, seqnames(genome), hits1)
```

4 Some precautions when using *matchPattern*

Improper use of *matchPattern* (or *countPattern*) can affect performance.

If needed, the *matchPattern* and *countPattern* methods convert their first argument (the pattern) to an object of the same class than their second argument (the subject) before they pass it to the subroutine that actually implements the fast search algorithm.

So if you need to reuse the same pattern a high number of times, it's a good idea to convert it *before* to pass it to the *matchPattern* or *countPattern* method. This way the conversion is done only once:

```
> library(hgu95av2probe)
> tmpseq <- DNAStringSet(hgu95av2probe$sequence)</pre>
 someStats <- function(v)</pre>
>
+
  {
+
       GC <- DNAString("GC")
       CG <- DNAString("CG")
+
       sapply(seq_len(length(v)),
+
               function(i) {
+
                   y <- v[[i]]</pre>
+
+
                   c(alphabetFrequency(y)[1:4],
                      GC=countPattern(GC, y),
+
+
                      CG=countPattern(CG, y))
               }
+
       )
+
+
  }
> someStats(tmpseq[1:10])
   [,1] [,2] [,3] [,4] [,5] [,6] [,7] [,8] [,9] [,10]
A
       1
            5
                  6
                        4
                              4
                                   2
                                         4
                                               5
                                                     9
                                                            2
С
      10
            5
                  4
                        7
                              5
                                   7
                                        10
                                               8
                                                     7
                                                           10
G
      6
            5
                  3
                        8
                              8
                                   6
                                         4
                                               5
                                                     4
                                                            4
Т
      8
           10
                                         7
                                               7
                 12
                        6
                              8
                                   10
                                                            9
                                                     5
GC
       2
                              3
                                   2
                                         2
                                               2
            1
                  1
                        4
                                                     1
                                                            1
                                               0
CG
       Ω
            Ω
                  Ω
                        2
                              1
                                    1
                                         Ω
                                                     Ω
                                                            Ω
```

5 Masking the chromosome sequences

Starting with Bioconductor 2.2, the chromosome sequences in a *BSgenome data package* can have built-in masks. Starting with Bioconductor 2.3, there can be up to 4 built-in masks per sequence. These will always be (in this order): (1) the mask of assembly gaps, (2) the mask of intra-contig ambiguities, (3) the mask of repeat regions that were determined by the RepeatMasker software, and (4) the mask of repeat regions that were determined by the Tandem Repeats Finder software (where only repeats with period less than or equal to 12 were kept).

For a given package, all the sequences will always have the same number of masks.

```
maskedwidth maskedratio active names
                                                                        desc
     30812367 5.384197e-01
                              TRUE AGAPS
                                                               assembly gaps
1
2
            5 8.737071e-08
                              TRUE
                                     AMB
                                                   intra-contig ambiguities
3
     16525661 2.887718e-01 FALSE
                                      RM
                                                                RepeatMasker
4
       872171 1.524044e-02 FALSE
                                     TRF Tandem Repeats Finder [period<=12]
all masks together:
  maskedwidth maskedratio
     47464316
                0.8293982
all active masks together:
  maskedwidth maskedratio
     30812372
                0.5384198
> chrM <- genome$chrM</pre>
> chrM
16569-letter MaskedDNAString object (# for masking)
seq: GATCACAGGTCTATCACCCCTATTAACCACTCACGGG...AGCCCACACGTTCCCCTTAAATAAGACATCACGATG
masks:
  maskedwidth maskedratio active names
                                                                        desc
                                                      assembly gaps (empty)
            0 0.00000e+00
                              TRUE AGAPS
1
2
            1 6.035367e-05
                              TRUE
                                     AMB
                                                   intra-contig ambiguities
3
          418 2.522784e-02 FALSE
                                      RM
                                                                RepeatMasker
4
            0 0.000000e+00 FALSE
                                     TRF Tandem Repeats Finder [period<=12]
all masks together:
  maskedwidth maskedratio
          419 0.02528819
all active masks together:
  maskedwidth maskedratio
            1 6.035367e-05
```

The built-in masks are named consistenly across all the BS genome data packages available in Bioconductor:

\mathbf{Name}	Active by default	Short description	Long description
AGAPS	yes	assembly gaps	Masks the big N-blocks that have been placed between the con
AMB	yes	intra-contig ambiguities	Masks any IUPAC ambiguity letter that was found in the conti
$\mathbf{R}\mathbf{M}$	no	Repeat Masker	Masks the repeat regions determined by the RepeatMasker soft
TRF	no	Tandem Repeats Finder	Masks the tandem repeat regions that were determined by the

Table 1: The built-in masks provided by the BS genome data packages.

When displaying a masked sequence (here a *MaskedDNAString* object), the *masked width* and *masked* ratio are reported for each individual mask, as well as for all the masks together, and for all the active masks together. The *masked width* is the total number of nucleotide positions that are masked and the *masked* ratio is the *masked width* divided by the length of the sequence.

To activate a mask, use the *active* replacement method in conjunction with the *masks* method. For example, to activate the RepeatMasker mask, do:

> active(masks(chrY))["RM"] <- TRUE > chrY

masks:							
maskedwidth	maskedratio	active	names				desc
1 30812367	5.384197e-01	TRUE	AGAPS			ä	assembly gaps
2 5	8.737071e-08	TRUE	AMB		intra	a-contig	g ambiguities
3 16525661	2.887718e-01	TRUE	RM				RepeatMasker
4 872171	1.524044e-02	FALSE	TRF	Tandem	Repeats	Finder	[period<=12]
all masks tog	ether:						
maskedwidth	maskedratio						
47464316	0.8293982						
all active ma	sks together:						
maskedwidth	maskedratio						
47337931	0.8271897						

As you can see, the masked width for all the active masks together (i.e. the total number of nucleotide positions that are masked by at least one active mask) is now the same as for the first mask. This represents a masked ratio of about 83%.

Now when we use a function that is *mask aware*, like alphabetFrequency, the masked regions of the input sequence are ignored:

> active(masks(chrY)) <- FALSE</pre>

> active(masks(chrY))["AGAPS"] <- TRUE</pre>

> alphabetFrequency(unmasked(chrY))

I	1	С	G	Т	М	H	R	W	S	
7886192	2 528578	39 52868	394 795	6168	0	(С	0	0	
У	<u>.</u>	K	V	Н	D	Η	3	Ν	-	
()	0	0	0	0	(308123	72	0	
4	-	•								
()	0								
> alphal	petFreque	ency(chr)	Y)							
A	С	G	Т	М	I	R	W	S	Y	Κ
7886192	5285789	5286894	7956168	0)	0	0	0	0	0
V	Н	D	В	N	ſ	-	+	•		

5

This output indicates that, for this chromosome, the assembly gaps correspond exactly to the regions in the sequence that were filled with the letter N. Note that this is not always the case: sometimes Ns, and other IUPAC ambiguity letters, can be found inside the contigs.

0

0

0

When coercing a MaskedXString object to an XString Views object, each non-masked region in the original sequence is converted into a view on the sequence:

> as(chrY, "XStringViews")

0

0

0

0

Views on a 57227415-letter DNAString subject views:

	\mathtt{start}	end	width	
[1]	10001	44821	34821	[CTAACCCTAACCCTAACCCTAAGGTCTCATTGAGGACAGATA]
[2]	94822	133871	39050	[CCCTCTTCCTGTCACGGCCCGGCCTAATGCAGACTCTAAAGG]
[3]	222347	226276	3930	[ACCCTAACCCTAACCCTAACCCTCCTTCCCTCGGCGCCATCC]
[4]	226352	1949345	1722994	[CCCGCTCCTCCCCTCGGGACCGTCATAACAAGAACCAAGATC]

This can be used in conjunction with the gaps method to see the gaps between the views i.e. the masked regions themselves:

```
> gaps(as(chrY, "XStringViews"))
```

To extract the sizes of the assembly gaps:

```
> width(gaps(as(chrY, "XStringViews")))
```

[1]	10000	50000	88475	75	183649	100	8260	50000
[9]	847	2052	50000	50000	50000	12393	1432	995
[17]	2486	2727	329	50000	25	808	534	2235
[25]	679	1523	1659	582	1078	899	908	1590
[33]	628	25	783	518	255	892	818	780
[41]	50000	267	38	1807	20	140	20	328
[49]	50000	1899	508	301	16000	30000000	50000	10000

Note that, if applied directly to chrY, gaps returns a MaskedDNAString object with a single mask masking the regions that are not masked in the original object:

> gaps(chrY)

```
1 26415048 0.4615803 TRUE
```

```
> alphabetFrequency(gaps(chrY))
```

А	С	G	Т	М	R	W	S
0	0	0	0	0	0	0	0
Y	K	V	Н	D	В	Ν	-
0	0	0	0	0	0 308	12367	0
+	•						
0	0						

In fact, for any MaskedDNAString object, the following should always be TRUE, whatever the masks are:

```
> af0 <- alphabetFrequency(unmasked(chrY))
> af1 <- alphabetFrequency(chrY)
> af2 <- alphabetFrequency(gaps(chrY))
> all(af0 == af1 + af2)
```

[1] TRUE

With all chrY masks active:

```
> active(masks(chrY)) <- TRUE</pre>
> af1 <- alphabetFrequency(chrY)</pre>
> af1
             С
                     G
                            Т
                                    М
                                            R
                                                   W
                                                           S
                                                                   Y
                                                                          Κ
     Α
2994088 1876822 1889305 3002884
                                    0
                                            0
                                                   0
                                                           0
                                                                   0
                                                                          0
                            В
     V
             Η
                     D
                                    Ν
                                                   +
     0
             0
                            0
                                                           0
                     0
                                    0
                                            0
                                                   0
> gaps(chrY)
57227415-letter MaskedDNAString object (# for masking)
masks:
 maskedwidth maskedratio active
     9763099
               0.1706018
                          TRUE
1
> af2 <- alphabetFrequency(gaps(chrY))</pre>
> af2
               С
                                Т
                        G
                                         М
                                                 R.
                                                          W
                                                                   S
      A
 4892104
         3408967
                  3397589
                          4953284
                                         0
                                                 0
                                                          0
                                                                   0
      Y
               Κ
                        V
                                Η
                                         D
                                                 В
                                                          Ν
                                                                   _
      0
               0
                        0
                                0
                                         0
                                                 0 30812372
                                                                   0
      +
               0
      0
> all(af0 == af1 + af2)
```

[1] TRUE

Now let's compare three different ways of finding all the occurences of the "CANNTG" consensus sequence in chrY. The Ns in this pattern need to be treated as wildcards i.e. they must match any letter in the subject.

Without the mask feature, the first way to do it would be to use the fixed=FALSE option in the call to matchPattern (or countPattern):

```
> Ebox <- "CANNTG"
> active(masks(chrY)) <- FALSE
> countPattern(Ebox, chrY, fixed=FALSE)
```

[1] 30953762

The problem with this method is that the Ns in the subject are also treated as wildcards hence the abnormally high number of matches. A better method is to specify the *side* of the matching problem (i.e. *pattern* or *subject*) where the Ns should be treated as wildcards:

```
> countPattern(Ebox, chrY, fixed=c(pattern=FALSE,subject=TRUE))
```

[1] 141609

Finally, countPattern being mask aware, this can be achieved more efficiently by just masking the assembly gaps and ambiguities:

```
> active(masks(chrY))[c("AGAPS", "AMB")] <- TRUE
> alphabetFrequency(chrY, baseOnly=TRUE)  # no ambiguities
```

 A
 C
 G
 T
 other

 7886192
 5285789
 5286894
 7956168
 0

 > countPattern(Ebox, chrY, fixed=FALSE)

[1] 141609

Note that some chromosomes can have Ns outside the assembly gaps:

```
> chr2 <- genome$chr2
> active(masks(chr2))[-2] <- FALSE</pre>
> alphabetFrequency(gaps(chr2))
         С
                     Т
   Α
               G
                          М
                                R.
                                      W
                                            S
                                                  Y
                                                        Κ
                                                              V
                                                                    Η
                                                                          D
                                                                                В
                                                                                      Ν
                                                                                            _
   0
         0
               0
                     0
                           0
                                 0
                                      0
                                            0
                                                  0
                                                        0
                                                              0
                                                                    0
                                                                          0
                                                                                0 2913
                                                                                            0
   +
   0
         0
```

so it is recommended to always keep the AMB mask active (in addition to the AGAPS mask) whatever the sequence is.

Note that not all functions that work with an *XString* input are *mask aware* but more will be added in the near future. However, most of the times there is a alternate way to exclude some arbitrary regions from an analysis without having to use *mask aware* functions. This is described below in the *Hard masking* section.

6 Hard masking

coming soon...

7 Injecting known SNPs in the chromosome sequences

coming soon...

8 Finding all the patterns of a constant width dictionary in an entire genome

The matchPDict function can be used instead of matchPattern for the kind of analysis described in the *Finding an arbitrary nucleotide pattern in an entire genome* section but it will be much faster (between 100x and 10000x faster depending on the size of the input dictionary). Note that a current limitation of matchPDict is that it only works with a dictionary of DNA patterns where all the patterns have the same number of nucleotides (constant width dictionary). See ?matchPDict for more information.

Here is how our runAnalysis1 function can be modified in order to use matchPDict instead of matchPattern:

```
+
      for (seqname in seqnames) {
          subject <- bsgenome[[seqname]]</pre>
+
          cat(">>> Finding all hits in strand", strand, "of chromosome", seqname, "...\n")
+
+
          mindex <- matchPDict(pdict, subject)</pre>
+
          matches <- extractAllMatches(subject, mindex)</pre>
          writeHits(seqname, matches, strand, file=outfile, append=append)
+
+
          append <- TRUE
          cat(">>> DONE\n")
+
+
      }
+ }
> runAnalysis2 <- function(dict0, outfile="")</pre>
+ {
      library(BSgenome.Celegans.UCSC.ce2)
+
      genome <- BSgenome.Celegans.UCSC.ce2
+
      seqnames <- seqnames(genome)</pre>
+
      runOneStrandAnalysis(dict0, genome, seqnames, "+", outfile=outfile, append=FALSE)
+
      runOneStrandAnalysis(dict0, genome, seqnames, "-", outfile=outfile, append=TRUE)
+
+ }
```

Remember that matchPDict only works if all the patterns in the input dictionary have the same length so for this 2nd analysis, we will truncate the patterns in ce2dict0 to 15 nucleotides:

```
> ce2dict0cw15 <- DNAStringSet(ce2dict0, end=15)</pre>
```

Now we can run this 2nd analysis and put the results in the "ce2dict0cw15_ana2.txt" file:

```
> runAnalysis2(ce2dict0cw15, outfile="ce2dict0cw15_ana2.txt")
```

```
Target: strand + of ce2 chromosomes chrI, chrII, chrIII, chrIV, chrV, chrX, chrM
>>> Finding all hits in strand + of chromosome chrI ...
>>> DONE
>>> Finding all hits in strand + of chromosome chrII ...
>>> DONE
>>> Finding all hits in strand + of chromosome chrIII ...
>>> DONE
>>> Finding all hits in strand + of chromosome chrIV ...
>>> DONE
>>> Finding all hits in strand + of chromosome chrV ...
>>> DONE
>>> Finding all hits in strand + of chromosome chrX ...
>>> DONE
>>> Finding all hits in strand + of chromosome chrM ...
>>> DONE
Target: strand - of ce2 chromosomes chrI, chrII, chrIII, chrIV, chrV, chrX, chrM
>>> Finding all hits in strand - of chromosome chrI ...
>>> DONE
>>> Finding all hits in strand - of chromosome chrII ...
>>> DONE
>>> Finding all hits in strand - of chromosome chrIII ...
>>> DONE
>>> Finding all hits in strand - of chromosome chrIV ...
>>> DONE
```

```
>>> Finding all hits in strand - of chromosome chrV ...
>>> DONE
>>> Finding all hits in strand - of chromosome chrX ...
>>> DONE
>>> Finding all hits in strand - of chromosome chrM ...
>>> DONE
```

9 Session info

```
> sessionInfo()
R version 4.4.1 (2024-06-14)
Platform: x86_64-pc-linux-gnu
Running under: Ubuntu 24.04 LTS
Matrix products: default
        /usr/lib/x86_64-linux-gnu/openblas-pthread/libblas.so.3
BLAS:
LAPACK: /usr/lib/x86_64-linux-gnu/openblas-pthread/libopenblasp-r0.3.26.so; LAPACK version 3.12.0
locale:
 [1] LC_CTYPE=en_US.UTF-8
                                LC_NUMERIC=C
 [3] LC_TIME=en_US.UTF-8
                                LC COLLATE=C
 [5] LC_MONETARY=en_US.UTF-8
                                LC_MESSAGES=en_US.UTF-8
 [7] LC_PAPER=en_US.UTF-8
                                LC NAME=C
 [9] LC_ADDRESS=C
                                LC_TELEPHONE=C
[11] LC_MEASUREMENT=en_US.UTF-8 LC_IDENTIFICATION=C
time zone: Etc/UTC
tzcode source: system (glibc)
attached base packages:
[1] stats4
                        graphics grDevices utils
                                                      datasets methods
              stats
[8] base
other attached packages:
 [1] BSgenome.Hsapiens.UCSC.hg38.masked_1.4.5
 [2] BSgenome.Hsapiens.UCSC.hg38_1.4.5
 [3] hgu95av2probe_2.18.0
 [4] AnnotationDbi_1.67.0
 [5] Biobase_2.65.0
 [6] BSgenome.Celegans.UCSC.ce2_1.4.0
 [7] BSgenome_1.73.0
 [8] rtracklayer_1.65.0
 [9] BiocIO_1.15.0
[10] Biostrings_2.73.1
[11] XVector_0.45.0
[12] GenomicRanges_1.57.1
[13] GenomeInfoDb_1.41.1
[14] IRanges_2.39.0
[15] S4Vectors_0.43.0
[16] BiocGenerics_0.51.0
```

	ed via a namespace (and not a	
	SparseArray_1.5.10	bitops_1.0-7
	RSQLite_2.3.7	lattice_0.22-6
	grid_4.4.1	fastmap_1.2.0
	blob_1.2.4	jsonlite_1.8.8
	Matrix_1.7-0	restfulr_0.0.15
[11]	DBI_1.2.3	httr_1.4.7
[13]	UCSC.utils_1.1.0	XML_3.99-0.17
[15]	codetools_0.2-20	abind_1.4-5
[17]	cli_3.6.3	rlang_1.1.4
[19]	crayon_1.5.3	bit64_4.0.5
[21]	cachem_1.1.0	DelayedArray_0.31.3
[23]	yaml_2.3.8	S4Arrays_1.5.1
[25]	tools_4.4.1	parallel_4.4.1
[27]	BiocParallel_1.39.0	memoise_2.0.1
[29]	GenomeInfoDbData_1.2.12	Rsamtools_2.21.0
[31]	SummarizedExperiment_1.35.1	curl_5.2.1
[33]	png_0.1-8	buildtools_1.0.0
[35]	vctrs_0.6.5	R6_2.5.1
[37]	matrixStats_1.3.0	KEGGREST_1.45.1
[39]	zlibbioc_1.51.1	bit_4.0.5
[41]	xfun_0.45	GenomicAlignments_1.41.0
[43]	sys_3.4.2	MatrixGenerics_1.17.0
[45]	knitr_1.47	rjson_0.2.21
[47]	maketools_1.3.0	compiler_4.4.1
[49]	RCurl_1.98-1.14	-